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14. ABSTRACT
The major objective of this project is to identify compounds that function as selective inhibitors of the essential glucose transporters of the parasite *Leishmania mexicana*. To identify such compounds, a cell growth assay was developed that can be employed in a high-throughput screen (HTS) for such inhibitors. This assay was employed in a scaling screen of the 2000 compound MicroSource Discovery Spectrum Collection and of the ~600,000 compound CBT library at St. Jude Children's Research Hospital with a Z-factor of 0.8. This screen yielded ~2800 compounds that qualified as 'hits' in the primary screen of the library, i.e. that inhibited growth of the *L. mexicana* line expressing the major glucose transporter designated LmxGT2 by >65%. These 2800 primary hits were subsequently subjected to a secondary screen in which dose-response curves were performed to asses the ability of each compound to differentially inhibit growth of parasites expressing LmxGT2 versus the human glucose transporter GLUT1. This secondary screen identified 14 compounds that exhibit an IC_{50} of <1 μM and a preferential inhibition of LmxGT2 cells by between 2-13 fold. 4 of these compounds showed significantly more potent inhibition of [^{3}H]D-glucose transport by LmxGT2 compared to GLUT1. An alternative and more direct secondary screen is now being developed that measures the ability of each primary hit to inhibit uptake of [^{3}H]D-glucose by LmxGT2.

15. SUBJECT TERMS
Leishmaniasis, drug development, selective inhibitors of essential parasite glucose transporters, high throughput screen, fluorescence cell growth assay, compound libraries

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Introduction:

Leishmania are parasitic protozoa that cause devastating diseases throughout much of the tropical and subtropical world (5), and infections of military personnel in the Middle East have become major medical problems for U.S. troops stationed in that region (see reports in DoD – GEISWeb: www.geis.fhp.osd.mil/GEIS/IDTopics/Leishmaniasis/LeishNavyPolicy.asp, entitled 'Leishmaniasis in Military Personnel Returning From Iraq' and the Militarycom web site: www.military.com/NewsContent/0,13319,FL_sick_032004,00.html, entitled 'Troops Being Treated For Leishmaniasis'). Drugs for treatment of leishmaniasis are generally expensive, toxic, and suffer from increasing occurrence of resistant parasites (7). Hence, identification of parasite-specific targets that could be exploited for development of novel drugs is of crucial importance. Our laboratory has demonstrated that the glucose transporters of *Leishmania mexicana* are essential for survival of *Leishmania* amastigotes inside mammalian host macrophages (2), suggesting that these important permeases could serve as critical targets for drug development. Furthermore, our laboratory has developed a cell-based assay (4) that can be employed in a high-throughput format to screen for compounds that selectively inhibit *Leishmania* but not human glucose transporters. The objective of this proposal is to first optimize this assay for use in a high-throughput screen (HTS) and to then employ the assay to screen large libraries of compounds for those that selectively inhibit the parasite glucose transporters. 'Hit' compounds that emerge from such screens will be further tested in glucose uptake assays to ensure that they are selective inhibitors of the parasite permeases. They will also be examined to determine their efficacy for growth inhibition of intracellular *Leishmania* parasites (amastigotes) and their 'therapeutic index', that is their relative toxicity for *Leishmania* parasites versus human cells. These latter screens will identify compounds able to inhibit parasite growth at concentrations that are not toxic to mammalian cells. Overall, the objective is to identify selective inhibitors of *Leishmania* glucose transporters that could serve as 'leads' for development of novel anti-leishmanial drugs.

Body:

To provide a comprehensive report of work to date, this report covers work done over the past ~ 3 years of the project. The material toward the end of the 'Body' summarize work done over the past year in which the validated high-throughput screen (HTS) was applied to a large chemical library of ~600,000 compounds and hits from primary and secondary screens were evaluated.

Foundation of the cell-based assay for use in the HTS. The principle of the cell-based assay is that *Leishmania* parasites that express a functional glucose transporter will grow in medium that contains glucose as a central carbon source but that does not contain an alternate carbon source, proline. Thus *L. mexicana* promastigotes (insect stage parasites that can be easily cultured in vitro) in which the glucose transporter genes have been deleted, the Δ*lmxgt* null mutants (2), are not able to grow in glucose-replete/proline-deficient (glucose +/proline -) medium (4). However, if these null mutants are complemented with the major glucose transporter gene from *L. mexicana*, *LmxGT2*, they will grow in glucose +/proline – medium. (*L. mexicana* encode three glucose transporter genes within a single 14 kb locus: *LmxGT1, LmxGT2, and LmxGT3* (1). The *LmxGT2* gene is the most highly expressed of these 3 genes in *L. mexicana* promastigotes. All three of these linked genes are deleted in the Δ*lmgt* null mutant.) Similarly, if the Δ*lmxgt* null mutant is complemented with the human glucose transporter gene, GLUT1, the parasites will also grow in glucose +/proline – medium. However, the growth of these complemented strains depends upon the function of the complementing glucose transporter. Thus, any compound that inhibits the complementing glucose transporter will strongly inhibit growth of the parasite line (4).

The HTS assay will screen for compounds that selectively inhibit growth of the null mutants expressing the LmxGT2 protein but that do not significantly inhibit growth of null mutants expressing the human GLUT1 protein. Such compounds will be selective inhibitors of LmxGT2 but not of GLUT1 and will thus selectively target the parasite glucose transporter. The screen will be performed first by identifying compounds in a library

that inhibit growth of the LmGT2-expressing null mutants. This subset of compounds will subsequently be rescreened against the null mutant that is expressing GLUT1 to identify those chemicals that do not inhibit this human transporter. Those compounds that are positive (inhibit parasite growth) in the first screen but negative (do not inhibit parasite growth) in the second screen will be candidates for selective inhibitors of *Leishmania* glucose transporters. These compounds will be further investigated, as detailed in the original proposal, to identify those that inhibit uptake of [^{3}H]glucose by LmxGT2 but not by GLUT1 (i.e. hit compounds).

Optimization of the fluorescence method employed for the cell-based assay. In developing any HTS, it is necessary to expend considerable effort optimizing the assay so that it can be used effectively in an automated high-throughput format (Assay Guidance Manual, http://www.ncgc.nih.gov/guidance/manual_toc.html). For initial optimization of the cell growth assay, we have examined two fluorescence assays for utility in the HTS format: i) the alamarBlue assay that monitors growth by the ability of live cells to reduce the dye alamarBlue, resulting in a change in the fluorescence spectrum; ii) the SYBR green assay that quantifies the amount of parasite DNA present by forming a fluorescent complex between the dye and DNA. To summarize, we have now determined that the SYBR green assay is superior to the alamarBlue method for several reasons. SYBR green gives a stronger fluorescence signal (excitation 485 nm, emission 528 nm) on a per cell basis than alamarBlue, SYBR green is cheaper on a per well basis than alamarBlue, the SYBR green method requires fewer steps in the assay, since fluorescence is read immediately after the dye-stop solution is added, and the SYBR green assay is an 'end point' assay in which cells are lysed at the time of dye addition. The advantage of an end point assay is that different plates can be read at different times after dye addition without adding the complication of further differential cell growth between plates that would affect the absolute values of the fluorescence signal. In contrast, this latter complication is a potential deficiency of the alamarBlue method in which cell growth is not stopped by addition of dye. For these reasons, we have chosen the SYBR green method for all our subsequent assay development studies. Thus the first accomplishment of the research program was to identify the optimal fluorescence assay to be employed for the HTS.

Test of assay quality employing control samples representing high (High), medium (Med), and low (Low) cell growth. In the first stage of development of an HTS, the assay method must be monitored for assay quality employing control samples. The details of recommended quality control tests are provided by the online Assay Guidance Manual published by Eli Lilly and company and the NIH Chemical Genomics Center (http://www.ncgc.nih.gov/guidance/manual_toc.html) that constitutes the 'gold standard' for development of an HTS. We have subsequently evaluated the SYBR green assay using first 96-well plates (data not shown but similar to that reported here) followed by miniaturization of the assay for 384-well plates that could be employed in a genuine HTS (data reported here). These control experiments have been performed on a robotics station (Synergy 4, Biotek, Winooski, VT) that performs all pipeting and fluorescence measurements in a fully automated format. In a critical evaluation of the method, a 'uniformity assay' was performed in which samples representing high cell growth (High, no added growth inhibitor), medium cell growth (Med, employing ~IC_{50} concentration of the drug phleomycin, 1.4 μM), and low cell growth (Low, employing a maximally inhibiting concentration of phleomycin, 1 mM) were arrayed in three 384-well plates in an interleaved pattern such that each well in the 384-well array received a High, Med, and Low sample among the three plates. These plates were read and the fluorescence values for all wells were measured. The purpose was to determine whether identical samples gave sufficiently reproducible readings across all wells in all three plates and whether there were any significant edge or position effects that create systematic errors in specific regions of the plates.

The results of this uniformity assay are presented in Table 1. This table summarizes the statistical criteria (Z'-factor, a statistical value that monitors assay quality (10); CV, coefficient of variation for each of the High, Med, and Low sets of samples; SD, standard deviation) and demonstrates that the experimental values for this assay are well within the range of the 'acceptance criteria', as defined in the Assay Guidance Manual.

Table 1. Summary of statistical variables calculated for the uniformity assay done with Δ*lmgt* cells expressing the LmxGT2 transporter. Cells were inoculated into 384-well plates at the indicated cell density and volume. Cell growth was terminated after 72 hr by addition of 5 µl SYBR green stop solution, and fluorescence (excitation 485 nm, emission 528 nm) was read.

PLATE	Initial cell density (ml^{-1})	Final vol/well	Inc. Time	Z'	CV Min %	CV mid %	CV Max %	Mean mid % inh	SD % mid inh
1	2.5×10^6	50 ul	72 hr	0.91	2.29	3.45	2.31	45.24	4.89
2	2.5×10^6	50 ul	72 hr	0.90	2.30	3.38	2.67	43.40	5.34
3	2.5×10^6	50 ul	72 hr	0.89	2.43	3.18	2.85	44.38	4.77
Acceptance criteria				**>0.40**	**< 20%**	**< 20%**	**< 20%**	**30-70%**	**< 20%**

An illustration of the Z'-factor is shown in Fig. 1, taken from reference (10). The Z'-factor (the Z-factor for an experiment performed only with High, Med, and Low control samples but with no library samples) is defined as the Separation Band/Dynamic Range and measures the signal compared to the variability of the data from well to well for both the High and Low control samples. The formula for calculating the Z'-factor is: $Z' = 1 - [(3SD_H + 3SD_L/(Av_H - Av_L)]$ where SD_H and SD_L represent the standard deviations of measurements for High and Low control samples and Av_H and Av_L represent the means of such measurements, respectively. A perfect assay would have a Z' value of 1.0, i.e. there would be no data variability, and the Separation Band would be equal to the Dynamic Range.

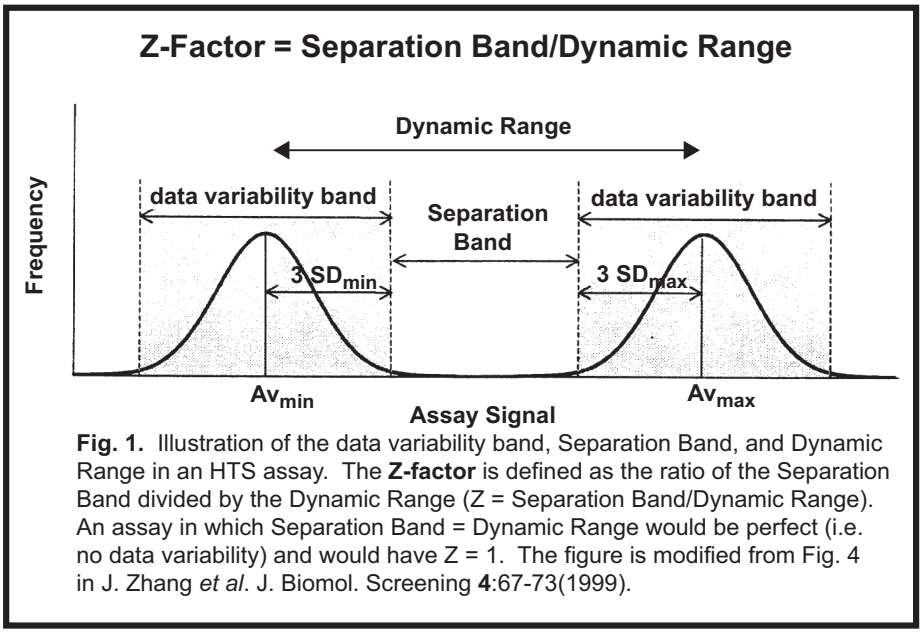

Fig. 1. Illustration of the data variability band, Separation Band, and Dynamic Range in an HTS assay. The **Z-factor** is defined as the ratio of the Separation Band divided by the Dynamic Range (Z = Separation Band/Dynamic Range). An assay in which Separation Band = Dynamic Range would be perfect (i.e. no data variability) and would have Z = 1. The figure is modified from Fig. 4 in J. Zhang *et al*. J. Biomol. Screening **4**:67-73(1999).

In practice, all assays exhibit some degree of data variability. The Z'-factor allows a quantitative measure of variability compared to signal strength and thus is a measure of assay quality. The Z' value must be >0.40 for an acceptable assay method (Assay Guidance Manual). Table 1 indicates that our assay generates Z' values of almost 0.90 for each of the three plates tested, revealing an extremely robust assay.

In addition, Figure 2 shows two plots, in different formats, of the data from one plate of this uniformity assay. These plots reveal that the variation is very low for fluorescence values of replicate High, Medium, and Low samples arrayed in different wells of the plate, and there are no significant edge or position effects within the plates that would contribute to false positive or false negative effects. Hence, the assay has passed the first set of criteria for a high quality screening method and is ready to go into production for screening of a small library. This so-called 'scaling screen' tests the assay in a screen of a real library, is an essential step in assay development (Assay Guidance Manual), and is a major objective of Specific Aim 1 of the original proposal.

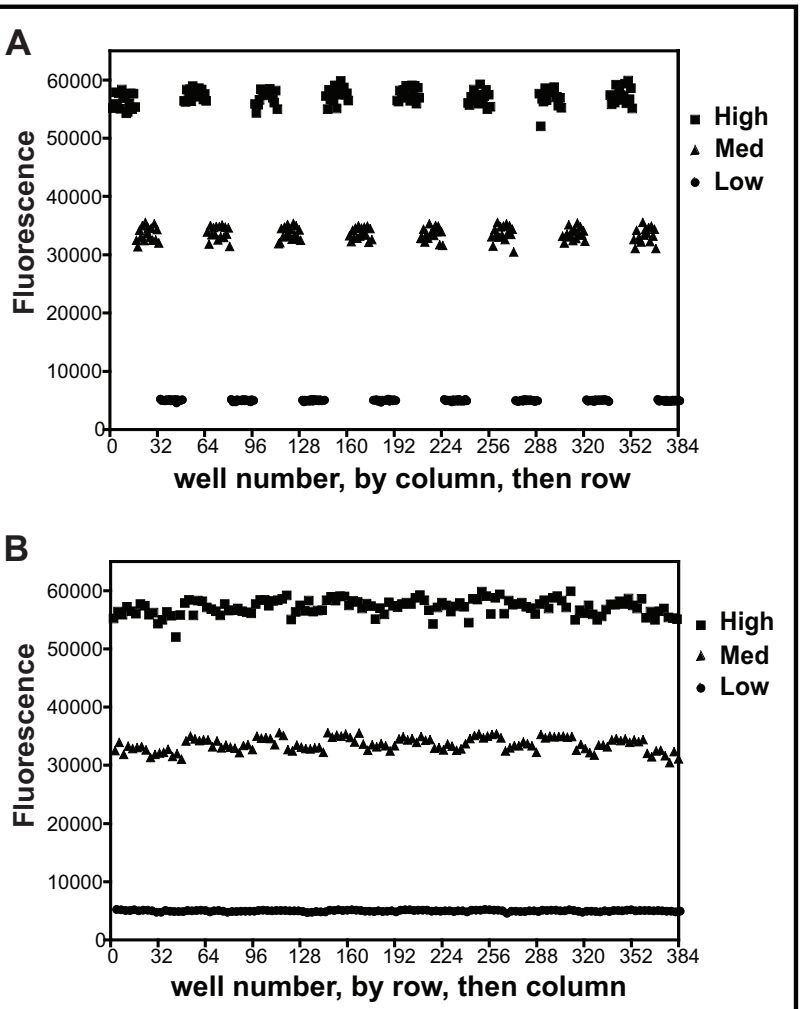

Fig. 2. Uniformity assay for control samples representing High (no growth inhibitor, squares), Med (~IC_{50} or 1.4 µM phleomycin, triangles), and Low (1 mM pleomycin, circles) growth conditions. Multiple samples of each type were arrayed across the plate as recommended in the Assay Guidance Manual. Results of one plate are shown here, but 3 such plates were prepared in parallel with interleaved patterns for the array such that each of the 384 wells received High, Med, and Low samples in one of the plates. Wells contained Δ*lmgt* null mutants expressing the LmGT2 transporter and were prepared in 50 µl final volumes as decribed in Table 1. Following 72 hr incubation, cell growth was stopped by addition of 5 µl SYBR green stop solution, and fluorescence was read (excitation 485 nm, emission 528 nm). The y-axis represents fluorescence units, and the x-axis represents individual wells plotted by column, then row in Part A or by row, then column in Part B. The results show high reproducibility for High, Med, and Low control samples and the absence of edge effects (systematically higher or lower signals near plate edges). The Z' value for this plate was 0.89.

Test of assay quality employing a 'scaling screen' of a small library of compounds. Following optimization of the SYBR green assay using control High, Med, and Low samples, it is necessary to test the assay against a small library to ensure that it is functioning robustly enough to employ in a genuine HTS. The principal criterion of acceptance employed at this stage is the Z-factor (10), a statistical value that is calculated similarly to the Z'-factor mentioned above but for the screen of the chemical library rather than for the measurements performed on control samples only. The formula for the Z-factor of a library screen is the same as that for the Z'-factor, except that SD_H and Av_H are replaced by SD_S and Av_S, the standard deviations and means for values determined for the library samples rather than the High control samples. As above, a Z-factor of >0.4 is considered to represent a screen of sufficient quality for advancement to the HTS stage.

The library employed in the scaling screen was the MicroSource Discovery Spectrum Collection, a library consisting of ~2000 compounds of which ~1000 are approved drug components, ~600 are natural products, and ~400 are other bioactive components. The library was constructed to have a wide range of biological activities and structural diversity. The library was screened in duplicate employing 13 384-well plates. High, Med, and Low control samples were also arrayed within each plate. Each well contained 20 µl of Δ*lmxgt* parasites, complemented with either LmxGT2 or GLUT1, suspended in DME-L medium (6) at an initial cell density of 5.6 x 10^6 cells/ml, and 25 µl of each compound, as a solution of 1% DMSO, to provide a final concentration of 10 µM compound. Parasites were grown for 72 hr at 26°C, after which 5 µl of stop solution containing a 100-fold dilution of commercial stock SYBR green (Sigma, St. Louis, MO) in 10% Triton X-100 was added to terminate cell growth and generate a fluorescence signal that was proportional to the cell density in each well. Fluorescence was read using the robotic platform, and the results were exported as an Excel file for data analysis.

Fig. 3 shows the results of the read from one of the 384-well plates. This figure demonstrates that the compounds from the library (data represented by solid circles) gave a range of inhibition of growth of parasites expressing LmxGT2. Most of the compounds resulted in little or no inhibition of growth, similar to data from

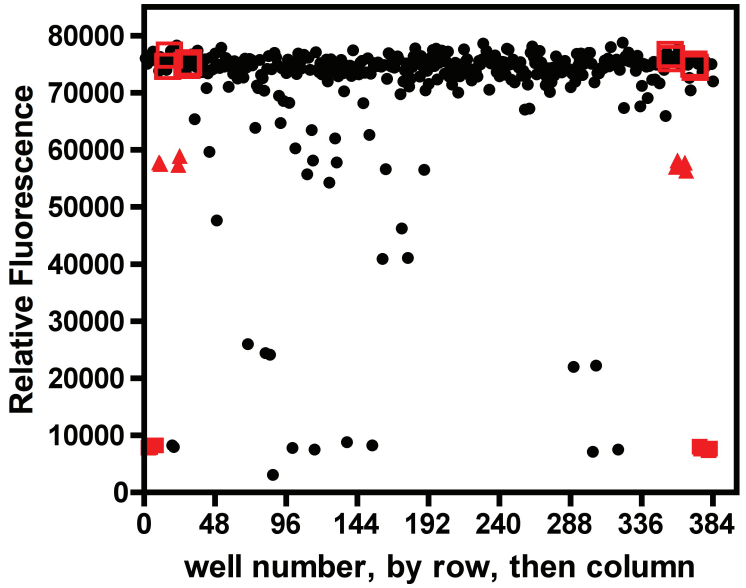

Fig. 3. The Δ*lmgt* null mutant expressing the LmGT2 transporter was grown for 72 hr in DME-L medium containing 5 mM glucose in the presence of compounds (2000) from the MicroSource Spectrum Collection (filled circles). The assay was performed in 384-well plates employing a 50 µl final volume in each well. The final concentration of each compound was 10 µM. In addition to library samples, controls containing no compound (High,open squares), 1.4 µM phleomycin (Med, solid triangles), or 1 mM phleomycin (Low, solid squares) were arrayed in each plate. Lysis buffer containing SYBR green was added at 72 hr, and the fluorescence signal (Relative Fluorescence, y-axis) representing cell growth was determined. The x-axis represents individual wells in the plate.

parasites incubated without any compound, and a limited number of compounds gave a high level of growth inhibition close to that of the Low control samples. Employing data from all 13 plates, the Z-factor was 0.84 for the library samples, and a Z'-factor calculated from the High and Low control samples was 0.92. These results indicate that the assay performs extremely robustly both when employing controls and when assaying a library of compounds. Hence, the assay is now ready to proceed in screening of larger compound libraries.

In addition to the primary function of establishing the quality of the assay method, the scaling screen might identify compounds that differently inhibit LmxGT2 compared to GLUT1. To determine whether any such compounds were detected in the screen, a subset of 140 compounds was identified that inhibited by 50% or more growth of parasites expressing LmxGT2. Subsequently, the fluorescence signal for parasites exposed to each of these 140 compounds was determined for the parasites expressing LmxGT2 (y-axis of Fig. 4) versus those expressing GLUT1 (x-axis of Fig 4). In this plot, compounds that inhibit LmxGT2 better than GLUT1 would be represented by spots below the line in the graph. (The line was determined by a least squares fit from control High, Medium, and Low samples only and represents data for a compound, phleomycin, that inhibits growth of both parasites lines equally.) It is clear from Fig. 4 that none of the 140 compounds inhibited growth of LmxGT2 expressing parasites significantly more than they inhibited growth of GLUT1 expressing parasites. Hence, this screen of a small library has not detected any compounds that preferentially inhibit the parasite glucose transporter. Screens of considerably larger libraries will constitute the next step in this program and will attempt to detect selective LmxGT2 inhibitors among a much larger group of compounds.

Screening of the Chemical Biology and Therapeutics (CBT) Library at St. Jude Children's Research Hospital. During the past year, we have performed a very large scale screen of the ~600,000 compound CBT library in collaboration with our colleagues in Dr. Kip Guy's laboratory at St. Jude Children's Research Hospital. This screen was performed using *L. mexicana* parasites expressing the LmxGT2 transporter. Primary hits (~2800) form this screen were identified as those that inhibited growth of the LmxGT2-expressing line by >65%. This cutoff value was determined using Receiver Operator Characteristics (3), a statistical method that

optimizes identification of the maximal number of true inhibitors while excluding the largest number of false positives (9).

These primary hits were subsequently subjected to a secondary screen to identify compounds likely to be selective inhibitors of LmxGT2 compared to GLUT1. This screen involved measuring dose-response (DR) curves for inhibition of growth of LmxGT2- and GLUT1-expressing Δ*lmxgt* parasites. Compounds were designated hits if they exhibited: 1) an IC_{50} for growth inhibition of LmxGT2-expressing parasites that was <1 μM, and 2) a differential IC_{50} for growth of LmxGT2- versus GLUT1-expressing parasites that was >2-fold. From this secondary screen, 14 compounds emerged that fell within these cutoff values; differential inhibition ranged from 2- to 13-fold lower IC_{50} for LmxGT2- versus GLUT1-expressing parasites. It was expected that the vast majority of the 2800 primary hits would not be selective inhibitors of LmxGT2. This is because the primary screen will identify all compounds that inhibit growth of *L. mexicana* parasites, and most of these inhibitors will function by 'off target' mechanisms, i.e. they will not be inhibitors of LmxGT2. However, we note that these primary hits do provide another 2800 compounds that may be of value as anti-leishmanial targets, although their mechanisms of action are currently unknown.

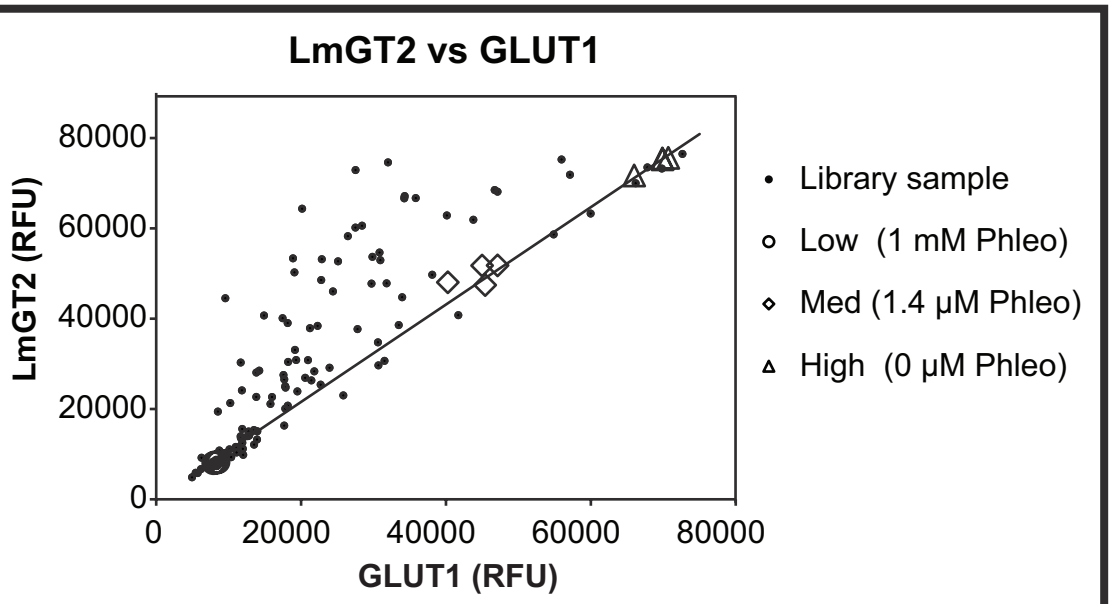

Fig. 4. The relative inhibition of growth of Δ*lmgt* null mutants expressing either LmGT2 or human GLUT1 was compared. The 140 compounds tested (filled circles; Library sample) were those from the MicroSource Discovery Collection that inhibited growth of LmGT2 expressing parasites by >50%. These compounds were retested for their ability to inhibit growth of parasites expressing GLUT1. The Relative Flurorescence Units (RFU), representing cell growth, were plotted for LmGT2 expressing parasites on the y-axis and for GLUT1 expressing parasites on the x-axis. The straight line was fitted to the data for phleomycin (Phleo; the Low, Med, and High data), a compound that inhibits growth of LmGT2 and GLUT1 expressing parasites equally. Compounds that selectively inhibit growth of LmGT2 expressing parasites over GLUT1 expressing parasites would fall below the straight line.

The 14 secondary hits were subsequently assayed for their ability to inhibit uptake of [^{3}H]D-glucose by LmxGT2 compared to GLUT1. At 10 μM concentration, 4 of the compounds showed significantly greater inhibition of uptake by LmxGT2 compared to GLUT1. Supplies of each compound are now being obtained that will allow us to measure dose-response curves for inhibition of glucose uptake for LmxGT2 versus GLUT1. These experiments should definitively identify inhibitors with significant differential inhibitory activity. Subsequently, multiple analogs of each compound not present in the original CBT library will be tested to determine whether there are related compounds with higher affinity of inhibition and with greater differential of activity.

Development of an Alternative Secondary Screen. One potential limitation of the current strategy Is that the secondary screen relies upon an 'indirect' measurement of differential inhibition: the ability to inhibit growth of LmxGT2- versus GLUT1-expressing parasites. It is possible that a more direct secondary screen that measures uptake of glucose directly will identify more accurately selective glucose transporter inhibitors. For

this reason, we are currently developing a radiolabeled glucose uptake assay that can be applied in a medium throughput format. This assay will then be employed to rescreen the 2800 primary hits and may detect additional promising hits. This assay is based upon incubation of parasites with [^{3}H]D-glucose followed by filtration on a membrane to remove parasites and the incorporated labeled glucose from the medium containing the labeled substrate. This approach has been employed routinely in a low throughput format to characterize transporters for glucose and other substrates In *Leishmania* parasites (8). Our modification will adapt this filtration assay to 96-well plates employing Millepore MultiScreen filter plates and the Millepore MultiScreen Vacuum Manifold. Since this format screens close to 100 samples per plate, it will be possible to screen all 2800 primary hits with relative ease. In addition, this medium throughput format will greatly facilitate further analysis oh hits, such as generation of dose-response curves for inhibition of glucose transport for each hit that emerges from the secondary screen.

Key Research Accomplishments:

- Determined that SYBR green represents a superior assay method, compared to alamarBlue, to monitor cell growth for a HTS. This method provides high fluorescence signal at low cost and has the advantage that it is an 'end point' assay.

- Established, using control samples representing High, Medium, and Low cell growth, that the cell growth assay performed both in 96-well and 384-well plates meets robust statistical criteria for use in a HTS, as outlined in the Assay Guidance Manual. In particular, a Z'-factor of close to 0.9 was obtained from these control experiments, far above the acceptable level of 0.4.

- Also established using 'uniformity assays' that the assay method does not suffer from position effects within plates or from unacceptable variation from plate to plate or from day to day when separate assays are performed.

- Performed in duplicate a 'scaling screen' of the MicroSource Discovery Spectrum Collection library of ~2000 compounds to further validate the assay in a screen of a small library. This screen generated a Z-factor of 0.84, indicating that the automated assay method is extremely robust.

- Searched for compounds in the MicroSource library that might differentially inhibit the parasite LmxGT2 transporter compared to the human GLUT1 glucose transporter. No such compounds were detected in this small-scale screen, indicating that screens of considerably larger libraries are warranted.

- The CBT library of ~600,000 compounds has been screened in high-throughput format, and ~2800 primary hits have emerged.

- A secondary screen of the primary hits was performed using a dose-response assay. Some 14 secondary hits were identified, and 4 of these hits emerged as selective inhibitors of LmxGT2 compared to GLUT1.

- A strategy has been developed for an improved secondary screen based upon direct measurement of inhibition of glucose uptake by each compound. This assay is now under development and will be applied to all of the primary hits.

Reportable Outcomes:

1. Seminar and research discussion, Department of Biochemistry, University of Iowa, January 2010.
2. Presentation of research results to the Portland Area Malaria Research Group in May 2010.

3. Research discussion, School of Pharmacy, University of Georgia, June 2010.

4. Research presentation to review committee, St Jude Children's Research Hospital, December 14, 2010.
5. Research presentation to Advinus Pharmaceuticals, January 6, 2011.
6. Research presentation to Merck, June 2, 2011.
7. Poster presentation summarizing results of screening at OHSU Research Forum, May 2012.

Conclusion:

The importance of the research accomplished during the first three years of this grant is that an assay for detection of compounds that selectively inhibit *Leishmania* glucose transporters has been developed. This assay has been optimized and shown to function robustly, employing various statistical and reproducibility criteria. The assay was subsequently employed in primary and secondary screens of a large chemical library, and 4 promising hits have emerged. A strategy for identification of further hits has also been developed.

'So what section'. The importance of this assay development is that it can be employed to identify 'hit' compounds that can be further explored for development of anti-leishmanial therapeutics. Initial hit compounds, those that selectively inhibit growth of parasites expressing LmxGT2 compared to GLUT1, will be further examined for their ability to inhibit growth of *L. mexicana* amastigotes within mammalian macrophages. Compounds that inhibit growth of *Leishmania* parasites without significant toxicity to mammalian cells can be further pursued as potential leads for anti-leishmanial drugs. An additional 2800 compounds have been identified that have ability to inhibit growth of *L. mexicana* in culture, providing a resource for identification of additional compounds with anti-parasitic activity.

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